

IN THE CLAIMS:

The following Listing of Claims replaces all prior Listings and versions of claims in the above-identified application.

Listing of Claims

1. (Original) A method to produce glucosamine or N-acetylglucosamine by fermentation, comprising:

a) culturing in a fermentation medium a microorganism which comprises at least one genetic modification that increases the activity of glucosamine-6-phosphate acetyltransferase; and

b) collecting a product produced from the step of culturing which is selected from the group consisting of glucosamine-6-phosphate, glucosamine, glucosamine-1-phosphate, N-acetylglucosamine-1-phosphate, N-acetylglucosamine-6-phosphate, and N-acetylglucosamine.

2. (Original) The method of Claim 1, wherein the genetic modification to increase the activity of glucosamine-6-phosphate acetyltransferase provides a result selected from the group consisting of: increased enzymatic activity of glucosamine-6-phosphate acetyltransferase; overexpression of glucosamine-6-phosphate acetyltransferase by the microorganism; reduced N-acetylglucosamine-6-phosphate product inhibition of the glucosamine-6-phosphate acetyltransferase; and increased affinity of glucosamine-6-phosphate acetyltransferase for glucosamine-6-phosphate.

3. (Original) The method of Claim 1, wherein the microorganism is transformed with at least one recombinant nucleic acid molecule comprising a nucleic acid sequence encoding the glucosamine-6-phosphate acetyltransferase.

4. (Original) The method of Claim 3, wherein the nucleic acid sequence encoding a glucosamine-6-phosphate acetyltransferase has at least one genetic modification which increases the enzymatic activity of the glucosamine-6-phosphate acetyltransferase.

5-6. (Cancelled)

7. (Original) The method of Claim 3, wherein the glucosamine-6-phosphate acetyltransferase has an amino acid sequence that is at least about 70% identical to an amino acid

sequence selected from the group consisting of: SEQ ID NO:30, SEQ ID NO:32 and SEQ ID NO:34, wherein the glucosamine-6-phosphate acetyltransferase has enzymatic activity.

8. (Original) The method of Claim 3, wherein the glucosamine-6-phosphate acetyltransferase has an amino acid sequence selected from the group consisting of SEQ ID NO:30, SEQ ID NO:32 and SEQ ID NO:34.

9. (Original) The method of Claim 3, wherein expression of the recombinant nucleic acid molecule is inducible.

10. (Original) The method of Claim 9, wherein expression of the recombinant nucleic acid molecule is inducible by lactose.

11. (Original) The method of Claim 10, wherein the microorganism further comprises a genetic modification to reduce inhibition of transcription induction by lactose.

12. (Original) The method of Claim 11, wherein the genetic modification comprises a partial or complete deletion or inactivation of a gene encoding a LacI repressor protein.

13. (Original) The method of Claim 1, wherein the microorganism further comprises at least one genetic modification that increases the activity of glucosamine-6-phosphate synthase.

14. (Original) The method of Claim 13, wherein the microorganism is transformed with at least one recombinant nucleic acid molecule comprising a nucleic acid sequence encoding the glucosamine-6-phosphate synthase.

15-16. (Cancelled)

17. (Original) The method of Claim 14, wherein the glucosamine-6-phosphate synthase comprises an amino acid sequence that is at least about 70% identical to an amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, and SEQ ID NO:20, wherein the glucosamine-6-phosphate synthase has enzymatic activity.

18. (Original) The method of Claim 14, wherein the glucosamine-6-phosphate synthase comprises an amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, and SEQ ID NO:20.

19. (Original) The method of Claim 14, wherein the glucosamine-6-phosphate synthase has a modification to reduce product inhibition of the glucosamine-6-phosphate synthase as compared to the wild-type glucosamine-6-phosphate synthase.

20. (Original) The method of Claim 19, wherein the glucosamine-6-phosphate synthase comprises an amino acid sequence selected from the group consisting of: SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, and SEQ ID NO:14.

21. (Original) The method of Claim 1, wherein the microorganism further comprises at least one genetic modification that decreases the activity of glucosamine-6-phosphate deaminase.

22. (Original) The method of Claim 21, wherein the genetic modification to decrease the activity of glucosamine-6-phosphate deaminase comprises a partial or complete deletion or inactivation of an endogenous gene encoding the glucosamine-6-phosphate deaminase in the microorganism.

23. (Original) The method of Claim 13, wherein the microorganism further comprises at least one genetic modification that decreases the activity of glucosamine-6-phosphate deaminase.

24. (Original) The method of Claim 23, wherein the genetic modification to decrease the activity of glucosamine-6-phosphate deaminase comprises a partial or complete deletion or inactivation of an endogenous gene encoding the glucosamine-6-phosphate deaminase in the microorganism.

25. (Original) The method of Claim 1, wherein the step of culturing includes the step of maintaining the carbon source at a concentration of from about 0.5% to about 5% in the fermentation medium.

26. (Original) The method of Claim 1, wherein the step of culturing is performed in a fermentation medium comprising yeast extract.

27. (Original) The method of Claim 1, wherein the step of culturing is performed in a fermentation medium comprising a carbon source selected from the group consisting of glucose, fructose, a pentose sugar, lactose and gluconic acid.

28. (Original) The method of Claim 27, wherein the pentose sugar is selected from the group consisting of ribose, xylose, and arabinose.

29. (Original) The method of Claim 1, wherein the step of culturing is performed in a fermentation medium comprising glucose and ribose.

30. (Original) The method of Claim 1, wherein the step of culturing is performed in a fermentation medium comprising glucose and gluconic acid.

31. (Original) The method of Claim 1, wherein the step of culturing is performed at a temperature of from about 25°C to about 45°C.

32. (Original) The method of Claim 1, wherein the step of culturing is performed at about 37°C.

33. (Original) The method of Claim 1, wherein the step of culturing is performed at a pH of from about pH 4 to about pH 7.5.

34. (Original) The method of Claim 1, wherein the step of culturing is performed at a pH of from about pH 6.7 to about pH 7.5.

35. (Original) The method of Claim 1, wherein the step of culturing is performed at a pH of from about pH 4.5 to about pH 5.

36. (Original) The method of Claim 1, wherein the microorganism is selected from the group consisting of bacteria and fungi.

37. (Original) The method of Claim 1, wherein the microorganism is selected from the group consisting of bacteria and yeast.

38. (Original) The method of Claim 1, wherein the microorganism is a bacterium from a genus selected from the group consisting of: *Escherichia*, *Bacillus*, *Lactobacillus*, *Pseudomonas* and *Streptomyces*.

39. (Original) The method of Claim 1, wherein the microorganism is a bacterium from a species selected from the group consisting *Escherichia coli*, *Bacillus subtilis*, *Bacillus licheniformis*, *Lactobacillus brevis*, *Pseudomonas aeruginosa* and *Streptomyces lividans*.

40. (Original) The method of Claim 1, wherein microorganism is a yeast from a genus selected from the group consisting of: *Saccharomyces*, *Candida*, *Hansenula*, *Pichia*, *Kluveromyces*, and *Phaffia*.

41. (Original) The method of Claim 1, wherein microorganism is a yeast from a species selected from the group consisting of: *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Candida albicans*, *Hansenula polymorpha*, *Pichia pastoris*, *P. canadensis*, *Kluyveromyces marxianus* and *Phaffia rhodozyma*.

42. (Original) The method of Claim 1, wherein the microorganism is a fungus from a genus selected from the group consisting of: *Aspergillus*, *Absidia*, *Rhizopus*, *Chrysosporium*, *Neurospora* and *Trichoderma*.

43. (Original) The method of Claim 1, wherein the microorganism is a fungus from a species selected from the group consisting of: *Aspergillus niger*, *A. nidulans*, *Absidia coerulea*, *Rhizopus oryzae*, *Chrysosporium lucknowense*, *Neurospora crassa*, *N. intermedia* and *Trichoderma reesei*.

44. (Original) The method of Claim 1, wherein the microorganism further comprises a genetic modification to increase phosphoglucosomerase activity in the microorganism.

45. (Original) The method of Claim 44, wherein the microorganism is transformed with a recombinant nucleic acid molecule comprising a nucleic acid sequence encoding the phosphoglucosomerase.

46. (Original) The method of Claim 44, wherein the phosphoglucosomerase comprises an amino acid sequence of SEQ ID NO:105.

47. (Original) The method of Claim 1, wherein the microorganism further comprises a partial or complete deletion or inactivation of phosphofructokinase in the microorganism.

48. (Original) The method of Claim 1, wherein the microorganism further comprises a genetic modification to increase the activity of glutamine synthetase.

49. (Original) The method of Claim 48, wherein the microorganism has been transformed with a recombinant nucleic acid molecule comprising a nucleic acid sequence encoding the glutamine synthetase.

50. (Original) The method of Claim 48, wherein the glutamine synthetase comprises an amino acid sequence of SEQ ID NO:89.

51. (Original) The method of Claim 1, wherein the microorganism further comprises a genetic modification to increase the activity of glucose-6-phosphate dehydrogenase.

52. (Original) The method of Claim 51, wherein the microorganism has been transformed with a recombinant nucleic acid molecule comprising a nucleic acid sequence encoding the glucose-6-phosphate dehydrogenase.

53. (Original) The method of Claim 51, wherein the glucose-6-phosphate dehydrogenase comprises an amino acid sequence of SEQ ID NO:95.

54. (Original) The method of Claim 1, wherein the microorganism further comprises a partial or complete deletion or inactivation of genes encoding enzymes responsible for glycogen synthesis in the microorganism.

55. (Original) The method of Claim 54, wherein the genes encoding enzymes responsible for glycogen synthesis comprise ADP-glucose pyrophosphorylase, glycogen synthase and a branching enzyme.

56. (Original) The method of Claim 1, wherein the genetic modifications do not inhibit the ability of the microorganism to metabolize galactose.

57. (Original) The method of Claim 1, wherein the step of collecting comprises recovering an intracellular product from the microorganism selected from the group consisting of: intracellular glucosamine-6-phosphate, glucosamine-1-phosphate, N-acetylglucosamine-6-phosphate, N-acetylglucosamine-1-phosphate, N-acetylglucosamine and glucosamine or recovering an extracellular product from the fermentation medium selected from the group consisting of: glucosamine and N-acetylglucosamine.

58. (Original) The method of Claim 1, further comprising a step selected from the group consisting of:

- a) purifying a product selected from the group consisting of glucosamine and N-acetylglucosamine from the fermentation medium;
- b) recovering a product selected from the group consisting of glucosamine-6-phosphate, glucosamine-1-phosphate, N-acetylglucosamine-6-phosphate and N-acetylglucosamine-1-phosphate from the microorganism;

c) dephosphorylating a product selected from the group consisting of glucosamine-6-phosphate and glucosamine-1-phosphate to produce glucosamine; and

d) dephosphorylating a product selected from the group consisting of N-acetylglucosamine-6-phosphate and N-acetylglucosamine-1-phosphate to produce N-acetylglucosamine

e) treating a product selected from the group consisting of N-acetylglucosamine, N-acetylglucosamine-6-phosphate and N-acetylglucosamine-1-phosphate to produce a glucosamine product selected from the group consisting of: glucosamine, glucosamine-6-phosphate and glucosamine-1-phosphate.

59. (Original) The method of Claim 54, wherein step (e) comprises hydrolyzing the product selected from the group consisting of N-acetylglucosamine, N-acetylglucosamine-6-phosphate and N-acetylglucosamine-1-phosphate, under acid and heat conditions or by enzymatic deacetylation.

60. (Original) The method of Claim 1, wherein N-acetylglucosamine produced by the fermentation method is recovered by precipitating N-acetylglucosamine-containing solids from the fermentation broth.

61. (Original) The method of Claim 1, wherein N-acetylglucosamine produced by the fermentation method is recovered by crystallizing N-acetylglucosamine-containing solids from the fermentation broth.

62-206. (Cancelled)

207. (Original) A method to produce glucosamine by fermentation, comprising:

a) culturing in a fermentation medium a microorganism which has been transformed with a recombinant nucleic acid molecule comprising a nucleic acid sequence encoding glucosamine-6-phosphate synthase, wherein expression of the recombinant nucleic acid molecule is controlled by a lactose induction, and wherein the step of culturing comprises:

i) growing the microorganism in the fermentation medium comprising glucose as a carbon source at a pH of from about pH 4.5 to about pH 7 and at a temperature of from about 25°C to about 37°C;

ii) inducing transcription of the nucleic acid sequence by addition of lactose to the fermentation medium in the absence of adding additional glucose to the medium;

iii) fermenting the microorganism after step (ii) in the presence of glucose at a pH of from about 4.5 to about 6.7 and at a temperature of from about 25°C to about 37°C; and

b) collecting a product produced from the step of culturing which is selected from the group consisting of glucosamine-6-phosphate and glucosamine.

208. (Original) The method of Claim 207, wherein a source of trace elements is added to step (iii) of fermenting.

209. (Original) The method of Claim 208, wherein the trace elements include iron.

210. (Original) The method of Claim 207, wherein step (ii) comprises growing the microorganism in the fermentation medium comprising glucose as a carbon source at a pH of about pH 6.9.

211. (Original) The method of Claim 207, wherein step (iii) comprises fermenting the microorganism after step (ii) in the presence of glucose at a pH of from about 4.5 to about 5.

212. (Original) The method of Claim 207, wherein step (iii) comprises fermenting the microorganism after step (ii) in the presence of glucose at a pH of about 6.7.

213. (New) The method of Claim 3, wherein the glucosamine-6-phosphate acetyltransferase has an amino acid sequence that is at least about 90% identical to an amino acid sequence selected from the group consisting of: SEQ ID NO:30, SEQ ID NO:32 and SEQ ID NO:34, wherein the glucosamine-6-phosphate acetyltransferase has enzymatic activity.

214. (New) The method of Claim 3, wherein the glucosamine-6-phosphate acetyltransferase has an amino acid sequence that is at least about 95% identical to an amino acid



sequence selected from the group consisting of: SEQ ID NO:30, SEQ ID NO:32 and SEQ ID NO:34, wherein the glucosamine-6-phosphate acetyltransferase has enzymatic activity.

215. (New) The method of Claim 14, wherein the glucosamine-6-phosphate synthase comprises an amino acid sequence that is at least about 90% identical to an amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, and SEQ ID NO:20, wherein the glucosamine-6-phosphate synthase has enzymatic activity.

216. (New) The method of Claim 14, wherein the glucosamine-6-phosphate synthase comprises an amino acid sequence that is at least about 95% identical to an amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, and SEQ ID NO:20, wherein the glucosamine-6-phosphate synthase has enzymatic activity.

217. (New) The method of Claim 14, wherein the glucosamine-6-phosphate synthase comprises an amino acid sequence that is at least about 95% identical to an amino acid sequence selected from the group consisting of: SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, and SEQ ID NO:14, wherein the glucosamine-6-phosphate synthase has enzymatic activity.

218. (New) A method to produce glucosamine or N-acetylglucosamine by fermentation, comprising:

- a) culturing in a fermentation medium a microorganism that expresses:
    - i) a recombinant nucleic acid molecule encoding a glucosamine-6-phosphate acetyltransferase; and
    - ii) a recombinant nucleic acid molecule encoding a glucosamine-6-phosphate synthase, wherein the glucosamine-6-phosphate synthase has a modification to reduce product inhibition of the glucosamine-6-phosphate synthase as compared to the wild-type glucosamine-6-phosphate synthase;
- wherein the microorganism comprises a partial or complete deletion or inactivation of phosphofructokinase; and

b) collecting a product produced from the step of culturing which is selected from the group consisting of glucosamine-6-phosphate, glucosamine, glucosamine-1-phosphate, N-acetylglucosamine-1-phosphate, N-acetylglucosamine-6-phosphate, and N-acetylglucosamine.

219. (New) A method to produce glucosamine or N-acetylglucosamine by fermentation, comprising:

- a) culturing in a fermentation medium a microorganism that expresses:
  - i) a recombinant nucleic acid molecule encoding a glucosamine-6-phosphate acetyltransferase that has an amino acid sequence that is at least about 95% identical to SEQ ID NO:30 and has glucosamine-6-phosphate acetyltransferase enzymatic activity; and
  - ii) a recombinant nucleic acid molecule encoding a glucosamine-6-phosphate synthase that has an amino acid sequence that is at least about 95% identical to SEQ ID NO:6 and has glucosamine-6-phosphate synthase enzymatic activity;

wherein the microorganism comprises a partial or complete deletion or inactivation of phosphofructokinase; and

b) collecting a product produced from the step of culturing which is selected from the group consisting of glucosamine-6-phosphate, glucosamine, glucosamine-1-phosphate, N-acetylglucosamine-1-phosphate, N-acetylglucosamine-6-phosphate, and N-acetylglucosamine.

220. (New) The method of Claim 219, wherein the glucosamine-6-phosphate acetyltransferase has an amino acid sequence of SEQ ID NO:30.

221. (New) The method of Claim 219, wherein the glucosamine-6-phosphate synthase has an amino acid sequence of SEQ ID NO:6.

222. (New) A method to produce glucosamine or N-acetylglucosamine by fermentation, comprising:

- a) culturing in a fermentation medium an *E. coli* that expresses:

- i) a recombinant nucleic acid molecule encoding a glucosamine-6-phosphate acetyltransferase that has an amino acid sequence of SEQ ID NO:30; and
- ii) a recombinant nucleic acid molecule encoding a glucosamine-6-phosphate synthase that has an amino acid sequence of SEQ ID NO:6;

wherein the *E. coli* comprises a partial or complete deletion or inactivation of *pfkA*; and

- b) collecting a product produced from the step of culturing which is selected from the group consisting of glucosamine-6-phosphate, glucosamine, glucosamine-1-phosphate, N-acetylglucosamine-1-phosphate, N-acetylglucosamine-6-phosphate, and N-acetylglucosamine.

223. (New) The method of Claim 219, wherein the *E. coli* further comprises a partial or complete deletion or inactivation of *nagA*, *nagB*, and *nagE*.

224. (New) The method of Claim 219, wherein the *E. coli* further comprises a partial or complete deletion or inactivation of *manXYZ*.

225. (New) The method of Claim 219, wherein the recombinant nucleic acid molecules of (a)(i) and (a)(ii) are inducible by lactose or galactose.

226. (New) The method of Claim 219, wherein the step of culturing is performed in a fermentation medium comprising glucose and fructose.